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**Author:** Janina Gospodarek, Aleksandra Nadgórska-Socha

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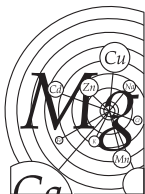
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## ORIGINAL PAPER

## CHEMICAL COMPOSITION OF BROAD BEANS (*VICIA FABA* L.) AND DEVELOPMENT PARAMETERS OF BLACK BEAN APHID (*APHIS FABAE* SCOP.) UNDER CONDITIONS OF SOIL CONTAMINATION WITH OIL DERIVATIVES\*

Janina Gospodarek<sup>1</sup>, Aleksandra Nadgórska-Socha<sup>2</sup>

<sup>1</sup>Department of Agricultural Environment Protection  
University of Agriculture in Krakow

<sup>2</sup>Department of Ecology  
University of Silesia in Katowice

## ABSTRACT

The indirect effect of oil derivatives, that is arising from the polluted soil through a host plant to the phytophagous fauna feeding on the plant, has not been investigated before. Hence, our goal has been to assess the effect of used engine oil, diesel fuel and petrol on the chemical composition of broad beans cultivated in soil polluted with these substances and on development parameters, such as fecundity, life span and intrinsic rate of natural increase, in a population of black bean aphid (*Aphis fabae* Scop.). Doses: 3 g kg<sup>-1</sup> (dose I) and 6 g kg<sup>-1</sup> (dose II) of an oil derivative per 1 kg of soil d.m. were used. Broad bean (*Vicia faba* L.) cv. White Windsor was chosen as the test plant used for studying the biology of aphids. When the experiment was completed, an analysis of selected elements (Zn, Fe, Mn, Ni, Cu, Pb, Cd, N, Ca and Mg) was conducted in leaves of broad bean. Soil pollution with petrol in dose I led to a significant decrease in the content of Ca, Mg, Fe, Mn, Zn, Ni and Cu, while causing a marked increase in the Pb content. The double dose of petrol (6 g kg<sup>-1</sup>) additionally contributed to diminishing the N content, whereas the concentrations of Ni and Cd increased significantly after its application. Soil pollution with diesel fuel, irrespective of a dose, caused a significant decline in the Ca, N, Zn, Ni and Cu concentrations and an increase in the Pb content. Additionally, the dose of 6 g kg<sup>-1</sup> led to an increase in the Mg, Fe and Cd content in broad bean plants. Soil pollution with used engine oil dosed at 3 g kg<sup>-1</sup> led to a reduction of the Ca, Mg, Fe, Mn, Zn and Cu content, while causing an increase in Pb and a slight increase in N concentrations. The double dose of engine oil caused a decline only in the Ca, Mn, Ni and Cu content. The effect of oil derivatives on the biology of black bean aphid was more pronounced in the second generation. All the analysed substances, except for engine oil in the lower dose, significantly decreased the intrinsic rate of natural increase in the population.

**Keywords:** oil derivatives, macro- and microelements, broad bean, *Aphis fabae* Scop.

dr hab. inż. Janina Gospodarek, Department of Agricultural Environment Protection, Agricultural University of Krakow, al. Mickiewicza 21, 31-120 Krakow

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## INTRODUCTION

Environmental pollution with oil derivatives becomes an increasingly serious ecological problem. These substances penetrate into waters and soils, where they negatively affect microbiological and biochemical properties of soil and consequently plant production. They also pose a hazard to animals and humans (ZIÓLKOWSKA, WYSZKOWSKI 2010). They may have different sources: machinery use in agriculture, application of insecticides based on petroleum oil, fertilisation of soil using municipal waste composts, discharge of used oils, spills from storage containers, damage to pipelines, road disasters or industry (NAJAR-RODRIGUEZ et al. 2008, SADEJ, NAMIOTKO 2010). Soil contamination with oil derivative entails far-reaching changes both in plant development and their chemical composition. The macro- and microelement content of plants changes and the level of heavy metals usually increases (LISTE, FELGENTREU 2005, WYSZKOWSKI, WYSZKOWSKA 2005, WYSZKOWSKI, ZIÓLKOWSKA 2009a,b, UJOWUNDU et al. 2011, RUSIN et al. 2015).

Arthropods are the most useful element of the natural environment for studies on the environmental impact of various kinds of pollutants. There are several underlying reasons, such as great diversity of arthropods, easy collection of specimens, affiliation to various trophic levels, but also a short period of generation development and consequently good adaptability to all kinds of changes. Aphids are distinguished among arthropods by a particularly high reproduction rate and even small changes in the composition of ingested sap may significantly affect their bionomics (HARRINGTON, STORK 1995). There is a considerable variety of responses of invertebrates to pollution with oil derivatives, depending on the concentration and type of a pollutant as well as invertebrate species and associated ability to metabolise toxic compounds (STROMBERG et al. 2004, COUCEIRO et al. 2007, SINGH et al. 2010). However, thus far research has focused on the soil and aquatic invertebrates prone to the direct contact with pollution components. The indirect effect of oil derivatives, that is arising from the polluted soil through a host plant to the phytophagous fauna feeding on the plant has not been investigated yet. Hence, the aim of the present study. Observations of the effect of a harmful substance on several levels create a more complex picture of a potential hazard they pose to subsequent links of the food chain, which has been documented, for example, in research on the influence of heavy metals on aphids and further on their predators or parasitoids (GREEN et al. 2003, GHABEISH 2014). Thus far, a soil-plant-herbivore model has been used to evaluate the environmental impact of heavy metals, such as: cadmium, zinc, nickel, lead and copper (MERRINGTON et al. 1997a,b, WINDER et al. 1999, GREEN et al. 2006, KAFEL et al. 2010), but also to assess the side effect of herbicide (LIPOK 2009). Our research goals were achieved through an observation of the effect of used engine oil, diesel fuel and petrol on the chemical composition of broad beans cultivated in soil polluted with these substances and on the develop-

ment parameters, such as fecundity, life span and intrinsic rate of natural increase, in a population of black bean aphid (*Aphis fabae* Scop.). A similar model, i.e. with broad bean applied as a test plant and black bean aphid as a herbivore insect, was used by LIPOK (2009) in his research on the side effects of phosphonate herbicides, and by CRAWFORD et al. (1995) concerning copper and cadmium. Another reason why black bean aphid was chosen as the research object was that it is a pest widespread in Europe and very harmful to many crops (including beetroots, broad bean and horse bean), each year causing great yield losses due to its direct harmfulness and plant viral diseases it carries (ŁUCZAK 1996). Its development on broad bean is most intensive, faster than on other host plants, therefore a potential influence of oil derivatives on the pest's bionomics would be easy to observe. Moreover, as demonstrated by MALALLAH et al. (1996), broad bean is a very useful plant bioindicator of oil pollution. Parameters, such as growth, levels of photosynthetic pigments, proteins, free amino acids, phenols, sugars, biomass, moisture and fatty acids, are highly differentiated under conditions of hydrocarbon-polluted soil. *Vicia faba* species was also used to assess the quality of aquatic and soil environments, including detection of mutagenic substances (GRANT et al. 1992, KANAYA et al. 1994).

## MATERIAL AND METHODS

A greenhouse pot experiment was conducted in 2013. Pots holding 9.8 kg d.m. of soil collected from agricultural land, from the 0-20 cm horizon, were used. The initial soil was degraded Chernozem formed from loess with pH close to neutral (pH in H<sub>2</sub>O = 6.56) and the humus content of 2.28%. According to the grain-size distribution, the soil was classified as silt clay deposit. The content of macro- and microelements (available forms) in the soil was: 15 mg N-NO<sub>3</sub>, 73 mg P, 115 mg K, 596 mg Ca, 65 mg Mg, 1.89 mg Cu, 17.22 mg Zn, 30.76 mg Mn, 132.61 mg Fe dm<sup>-3</sup> of soil. Total amounts of heavy metals were 0.41 mg Cd, 16.3 mg Ni, 22 mg Pb kg<sup>-1</sup> soil dry mass. The analyses were performed in the laboratory of the Regional Chemical and Agricultural Station in Kraków. The soil was polluted with unleaded petrol (P), diesel fuel (DF) and used engine oil (EO). The applied doses were 3 g (dose I) and 6 g (dose II) of an oil derivative per 1 kg of soil d.m. Basic fertilisation, same for all objects: 0.7 g N (as NH<sub>4</sub>NO<sub>3</sub>); 0.35 g P (as KH<sub>2</sub>PO<sub>4</sub>); 1g K (as KCl) per pot, was applied prior to adding a pollutant to the soil. Weighted batches of soil (separate for each pot) were crumbled carefully and a thin layer of soil was spread on a foil mat. Subsequently, it was sprinkled with fertiliser solutions by means of a hand sprinkler and the soil was mixed thoroughly. Then a measured amount of an appropriate oil derivative was applied by uniform pouring onto the surface of the soil using a laboratory pipette, after which the soil was again mixed several times for even distribution of the pollutant

in the whole soil volume. The soil thus prepared was transferred to pots. Broad bean seeds were sown a week later.

Broad bean (*Vicia faba* L.), cv. White Windsor, was chosen as the test plant for studying the biology of black bean aphid (*Aphis fabae* Scop.). After emergence, the plants were thinned and 5 plants were left per pot. The pot experiment was set up in five replications. When the plants grew to about 30 cm of height (i.e. 6 weeks after sowing), studies on aphid biology started. The pest development was followed in isolating containers, 12 cm in diameter and 20 cm high, made of densely woven airy fabric, placed on broad bean leaves (at the same height on each plant to avoid leaf age influence on the pest biology). One container covered only one, whole single broad bean leaf, used finally for investigating the development of a single aphid female. The investigations were conducted on *Aphis fabae* Scop. aphids from own culture of the Agricultural Environment Protection Department, maintained on the same host plant, i.e. broad bean cv. White Windsor.

Three wingless females were put into each container and removed after giving birth to the first larvae. A single larva was left in each isolating container. When the larvae reached sexual maturity, their fecundity was counted every day for 13 days, and subsequently every 2 to 3 days, but each newborn larva was removed (except for the first larvae which were removed to new isolating containers for studying fecundity and biology of the next generation). Two containers were placed on each test plant to study the bionomics of the 1<sup>st</sup> generation (the total of 50 containers per object) to ensure an adequate final number of females to watch the bionomics of *A. fabae*. This procedure was applied because some of the initial females placed artificially with a brush always die before giving birth to larvae which are the object of research on *A. fabae* bionomics. Other containers, additionally 2 on each plant, were put on the test plants (on other branches of broad bean plants) to investigate the bionomics of the 2<sup>nd</sup> generation of aphids, which appears before the 1<sup>st</sup> generation development is completed.

The female fecundity and duration of their development were counted for 30 females of each generation. Parameters of the population development were counted using the methodology developed by WYATT and WHITE (1977):

$$r_m = (0.738 \cdot \ln(M_d)) d^{-1},$$

where:  $r_m$  – the intrinsic rate of natural increase in a population,  $d$  – the duration of the pre-reproductive period (the time from birth until producing offspring by females),  $M_d$  – the number of larvae born during the reproduction period equal to  $d$ .

When the experiment was completed, an analysis of selected elements (Zn, Fe, Mn, Ni, Cu, Pb, Cd, N, Ca and Mg) was conducted in leaves of broad bean on which aphids were feeding. In order to determine metal concentrations, plant material was washed in tap and distilled water and dried at 105°C. A portion of 0.25 g dried plant material was digested with 5 mL of

HNO<sub>3</sub> at 120°C and then diluted to 10 mL with deionized water. Next, the content of the metals was measured using flame absorption spectrometry (ICE 3500). The quality of the analytical procedure was checked using reference material (Certified Reference Material CTA-OTL-1 Oriental Tobacco Leaves) with the same quantities of samples. The levels of N were measured with a CNS analyzer (Variomax CNS, Elementar Analysensysteme GmbH, Germany). The experiments were performed in three replicates.

Statistical computations were conducted with Statistica 10.0 software. The significance of differences between the means was examined using one-way analysis of variance. The means were differentiated using the Fisher's LSD test (for aphids) or *t*-Tukey's test (for the elemental content) at the significance level  $\alpha = 0.05$ . Standard error of the mean ( $\pm$ SE) and standard deviation ( $\pm$ SD) were also computed for selected parameters.

## RESULTS AND DISCUSSION

Soil contamination with diesel fuel caused a decrease in the nitrogen concentration in broad bean aerial parts, but the dose of 3 g kg<sup>-1</sup> had a more limiting effect (Figure 1). Considering the other oil derivatives, only the higher dose of petrol led to a noticeable (by ca 23%) decline in the N content, whereas the lower dose of engine oil revealed even a slight (by 7%) positive influence on this element's content. WYSZKOWSKI and WYSZKOWSKA (2005) obtained very similar results for maize. A dose of 3 g kg<sup>-1</sup> of diesel fuel at the simultaneous application of sawdust caused a decline in the nitrogen concentration in the aerial parts by about 35%, whereas the dose of 6 g kg<sup>-1</sup> in the same fertilisation variant decreased the same value by only 27%. In the other fertilisation variants, the above authors observed either an increase or a slight decrease in the N content in maize aerial parts. On the other hand, in the case of oats, the above diesel fuel doses led to an increase in nitrogen concentrations in the aerial parts of maize. Changes in the nitrogen content in lupine plants under the influence of the contamination with diesel fuel were strongly conditioned by the type of soil and applied nitrogen fertilisation (WYSZKOWSKI et al. 2004). In light loamy sand soil, increasing doses of diesel fuel with simultaneous nitrogen fertilisation caused a decrease in the N content at the dose of 3.38 g kg<sup>-1</sup>, but an increase to the control level at the dose of 5.07 g kg<sup>-1</sup>. Soil contamination with oil derivatives leads to the disturbance of carbon to nitrogen ration, which may finally cause a change in the course of nitrogen transformations in soil, but also affect the intensity of ammonification and nitrification processes (ADAM, DUNCAN 2003).

In our experiment, both the higher and lower doses of the applied oil derivatives caused a significant decrease in the calcium content in broad bean leaves (Figure 1). The highest (almost twofold) decrease in this element's content was noted in the object where the soil was polluted by the



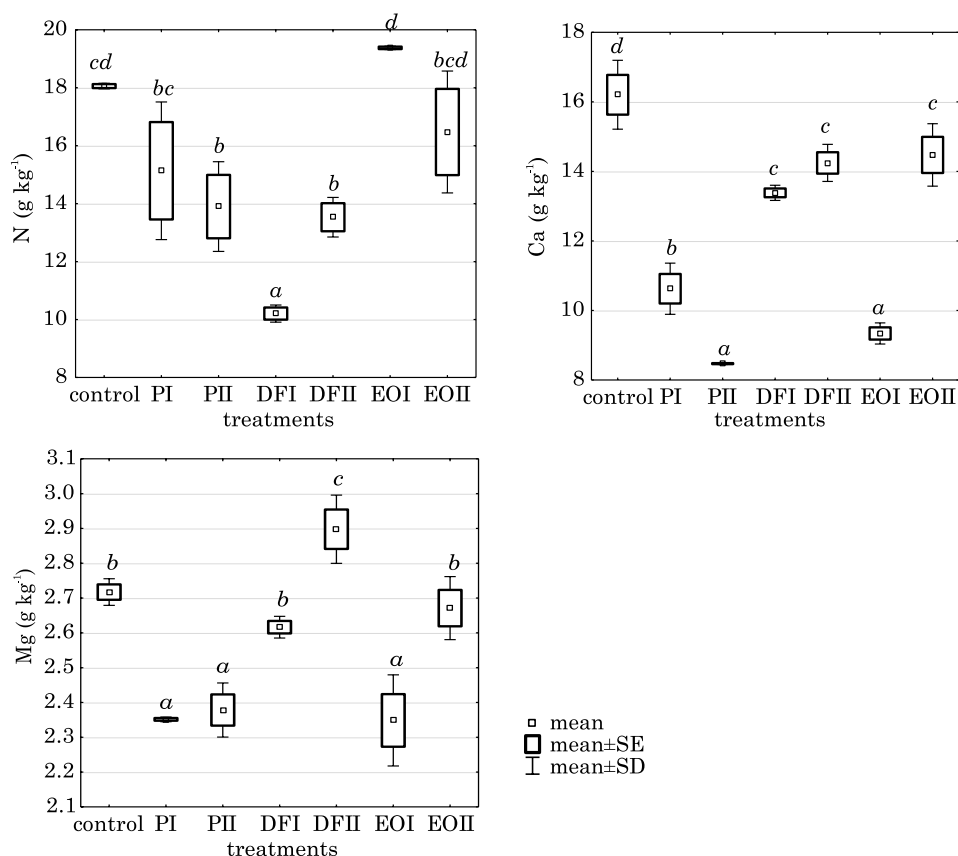


Fig. 1. Content of nitrogen, calcium and magnesium in broad bean ( $\text{g kg}^{-1}$  d.m.): P – unleaded petrol, DF – diesel oil, EO – used engine oil, I – dose  $3 \text{ g kg}^{-1}$ , II – dose  $6 \text{ g kg}^{-1}$ . Means followed by the same letters did not differ significantly at  $\alpha = 0.05$  according to the *t*-Tukey test

higher dose of petrol. A considerable decline in the Ca content also occurred due to the lower doses of engine oil and petrol. On the other hand, the higher dose of engine oil and both doses of diesel fuel caused diminishing of the Ca content by between 10 and 17%. In the research conducted by WYSZKOWSKI and WYSZKOWSKA (2005), diesel fuel doses of 3 and  $6 \text{ g kg}^{-1}$  generally caused a decline in the calcium content in maize and oats aerial parts, fluctuating from a few to a dozen or so per cent, depending on the applied nitrogen fertilisation and sawdust. Similarly to our experiment, in some objects, the higher dose of diesel fuel ( $6 \text{ g kg}^{-1}$ ) limited the Ca content to a lesser extent than half that dose ( $3 \text{ g kg}^{-1}$ ).

Both doses of petrol and the lower dose of engine oil caused a similar decline in the magnesium content, i.e. about 13%, whereas the higher dose of engine oil and the lower one of diesel fuel did not affect this element (Figure 1). For the diesel fuel dose of  $6 \text{ g kg}^{-1}$  soil d.m., an approximately 6% increase in

the Mg content was observed in broad bean aerial parts. A similar increase in the Mg content of maize shoots (by about 10%) under the influence of the same dose of diesel fuel (6 g kg<sup>-1</sup>) was documented by WYSZKOWSKI and WYSZKOWSKA (2005), but only when nitrogen foliar feeding (125 mg N kg<sup>-1</sup>) had been applied. The Mg content in lupine aerial parts was also increasing with the increasing doses of diesel fuel (WYSZKOWSKI et al. 2004), whereas petrol doses of 2.5 and 5.0 g kg<sup>-1</sup> caused a decrease in Mg in oat plants by about 5 - 6% (WYSZKOWSKI, ZIÓŁKOWSKA 2009a).

The unfavourable effect on the iron content in broad bean leaves was visible when the soil was contaminated with petrol (a decline by 64% for the lower dose and 52% for the higher one). Also, the lower dose of engine oil contributed to a decrease in this element's concentration in broad bean, whereas a significant increase in the Fe content (by *ca* 1/3) was effected by soil pollution with the higher dose of diesel fuel (Figure 2). ODJEGBA and ATEBE (2007) obtained similar results for used engine oil, although the increase in

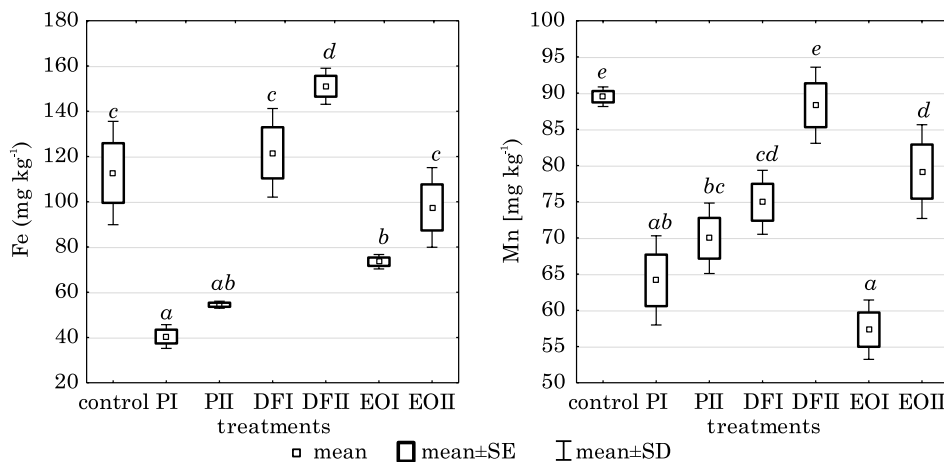


Fig. 2. Content of iron and manganese in broad bean (mg kg<sup>-1</sup> d.m.). Means followed by the same letters did not differ significantly at  $\alpha = 0.05$  according to the *t*-Tukey test; see Fig. 1 for symbols

the contamination level (1, 2, 3, 4 and 5%) resulted in a proportional decrease in Fe concentrations in *Amaranthus hybridus* L. shoots.

Almost all the applied types of pollutants caused a significant decrease in the Mn content in broad bean leaves, with the highest noted at the lower dose of engine oil. Only the higher dose of diesel fuel did not affect markedly this element's content. Concentrations of this metal may fluctuate in plants growing in soil polluted with oil derivatives. Research on the effect of crude oil on microelement content in jojoba (*Simmondsia chinensis*) plants evidenced an increase in the manganese content under the influence of this factor (SHUKRY et al. 2003). An increase in manganese concentrations in *Xanthosoma sagittifolium* plants, but a decline in *Manihot esculenta*, *Vernonia amygdalina*



and *Talinum triangulare* under the influence of oil derivatives was demonstrated by NWAICHI et al. (2014).

Pollution with oil derivatives may also result in increasing content of some heavy metals in soil and subsequently in plants, not only because of their supply from soil (ALMUZAINI, JACOB 1996, SANTOS-ECHEANDIA et al. 2008), but also because oil derivatives modify the conditions of the uptake of heavy metals by plants (MING NIE et al. 2010, XIAO-WEN et al. 2014). Heavy metals in plants may in turn influence aphid development (CRAWFORD et al. 1995, GOSPODAREK 2005, GORUR 2007, 2009), but they can also be accumulated in aphids and thus enter the trophic chain (CRAWFORD et al. 1995, MERRINGTON et al. 2001). Almost all the applied doses of the oil derivatives investigated in our research contributed to a significant increase in the lead content in broad bean leaves, although the lower doses increased Pb concentrations more than the double ones (Figure 3). The lead content in the object polluted with engine oil dosed at 6 g kg<sup>-1</sup> did not differ significantly from this metal's content in the control. Lead is accumulated mainly in plant roots and is characterised by low mobility (KUMARI et al. 2013), hence under the worse growth conditions for plants at higher doses of oil derivatives, Pb translocation to aerial plant organs may be inhibited. An increase in lead concentrations in broad bean leaves due to soil contamination with oil derivatives was also reported by RUSIN et al. (2015). As demonstrated by BENKA-COKER and EKUNDAYO (1995) and OKONOKHUA et al. (2007), soil pollution with oil derivatives contributes to increased lead concentrations, which may explain elevated concentrations of this metal in plant leaves. UJOWUNDU et al. (2011) revealed that oil derivative compounds cause an increase in cadmium and lead concentrations in soil, but a decrease in copper and zinc content. It has been confirmed by the results of the current investigations. The content of zinc, nickel and copper in most cases declined under the influence of the applied oil derivatives. At the same time, soil contamination with petrol dosed at 3 g kg<sup>-1</sup> was the most severely limiting factor, and a similar effect was produced on nickel by used engine oil dosed at 6 g kg<sup>-1</sup>. The higher dose of petrol caused an increase in the Ni content in broad bean leaves by about 70%, but did not notably affect the Cu content. No statistically proven effect of the higher dose of engine oil on the Zn concentration was observed. The cadmium content noted in broad bean aerial parts was below the detection level in most objects except the soils contaminated with higher doses of petrol and diesel fuel.

Mean fecundity of the first generation of black bean aphid wingless females fluctuated between the objects from 32.75 to 44.50 larvae per female (Table 1). No significant effect of the lower doses of the oil derivatives on this biological parameter was recorded, also the higher dose of engine oil only slightly decreased the number of larvae produced by a single female (on average by about 5.3 individuals). On the other hand, the dose of 6 g kg<sup>-1</sup> of petrol and diesel fuel markedly declined fecundity of *A. fabae* wingless females: by 10.89 larvae for petrol and by 9.37 for diesel fuel. Fecundity in the second generation was visibly lower (from 18.14 to 31.19 larvae per female). A nega-

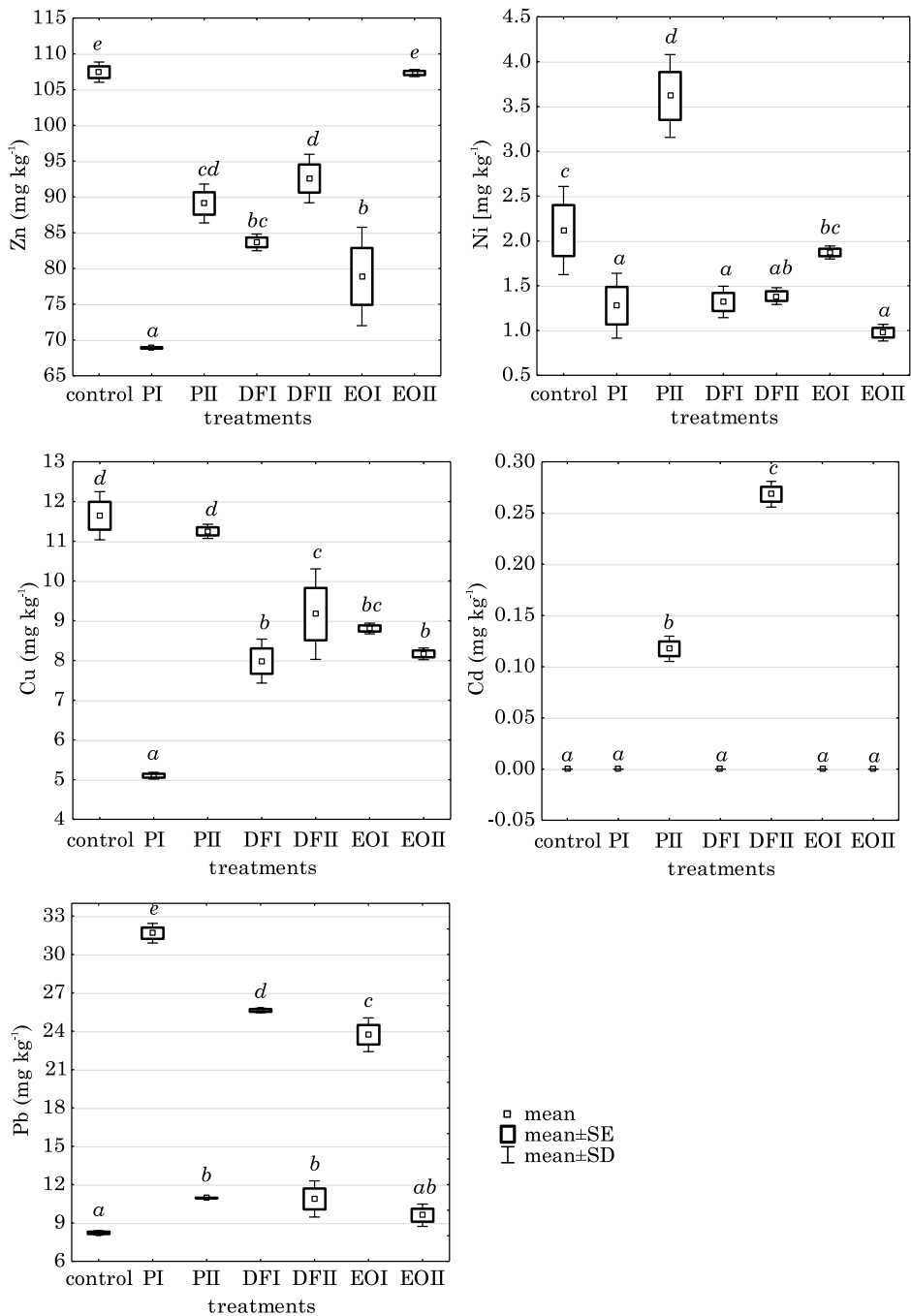


Fig. 3. Content of zinc, nickel, copper, cadmium and lead in broad bean (mg kg<sup>-1</sup> d.m.). Means followed by the same letters did not differ significantly at  $\alpha = 0.05$  according to the *t*-Tukey test; see Fig. 1 for symbols

Table 1

Fecundity and life span of black bean aphid *Aphis fabae* Scop. on broad bean growth in soil polluted with oil derivatives

Details	Mean fecundity (No. of larvae per one female)		Mean life span (days)	
	I	II	I	II
Control	43.64b*	28.34bc	25.18ab	21.21a
PI	41.86ab	23.46ab	28.33ab	20.42a
PII	32.75a	28.13bc	23.06a	21.04a
DFI	42.25ab	18.14a	30.06b	18.21a
DFII	34.27a	23.10ab	24.36ab	21.55a
EOI	44.50b	31.19c	30.41b	22.75a
EOII	38.34ab	21.48a	22.40a	20.31a

\* Means followed by the same letters in columns did not differ significantly at  $\alpha = 0.05$  according to the LSD test; see Fig. 1 for symbols

tive effect of the higher dose of engine oil and the lower dose of diesel fuel became apparent in this case. On the other hand, similarly to the first generation fecundity was the highest in the object contaminated with the lower dose of engine oil (EOI) among all the investigated objects, including the control. The available literature lacks information about the effect of soil contamination with oil derivatives on the development parameters of herbivores feeding on plants growing in polluted soil. The few studies which dealt with the effect of oil derivatives on herbivores were conducted in the aquatic environment and suggested that this invertebrate group was most sensitive to oil spills (KOTTA et al. 2007). On the other hand, some springtails are soil herbivorous invertebrates. Numerous investigations have been conducted on these animals, documenting their high sensitivity to the presence of oil derivatives in the soil environment, which showed as an increased lethality rate, shorter life span and inhibition of development (KIREEVA et al. 2005, ANTONIOLLI et al. 2013). However, in this case, the type of exposure is different and results mainly from the direct contact of integuments with these pollutants, and not via an indirect effect through the host plant.

Some potential causes of changes observed in development parameters of black bean aphid may be inferred indirectly through the analysis of tested pollutants' effect on the plant chemical composition. Low doses of oil derivative substances are not toxic to soil and consequently to plants; moreover, plants reveal highly diverse sensitivity to their presence (WYSZKOWSKI, ZIÓŁKOWSKA 2009b). Therefore, the high fecundity of *A. fabae* females in the EOI object may be explained as the absence of any negative effect of such pollutants on broad bean's growth and composition. Organic substances supplied to soil may beneficially affect some pests. KARUNGI et al. (2006) demonstrated that organic fertilisation (composted or not composted organic waste) favour

*A. fabae* aphid's occurrence on beans. Similarly, farmyard manure favoured black bean aphid's presence on horse bean (SADEJ, SADEJ 2001). Nitrogen concentrations in broad bean shoots growing in soil polluted with 3 g kg<sup>-1</sup> of engine oil was 7% higher than in the control. On the other hand, the lowest nitrogen content was determined in plants growing in soil polluted with the lower dose of diesel fuel.

Here again, the lowest fecundity of *A. fabae* females was noted in the second generation. As it is known, aphid occurrence is positively correlated with the content of nitrogen compounds in the host plant. A very high positive correlation ( $r = 0.783 - 0.933$ ) between the presence of *Metopolophium dirhodum* Walker aphid and chlorophyll content in spring wheat was reported by HONĚK and MARTINKOVA (2002), who attributed this fact to the increase in the amount of nitrogen compounds and assimilation products in plants rich in chlorophyll. DAVIES et al. (2004) observed a similar effect when analysing the effect of growing N doses on the occurrence of *Aphis gossypii* Glover aphids on chrysanthemums. Bean inoculation with *Rhizobium*, leading to an elevated nitrogen content, entailed greater *A. fabae* density (NALUYANGE et al. 2014). Decreased fecundity on PII and DFII objects in the first generation and EOII in the second generation may be also partly connected with the lowering of the N content in broad bean aerial parts in this objects. Moreover, a considerable decrease in calcium concentration was observed in PII object. Admittedly, insects' requirement for calcium is much lower than in vertebrates, although its excess or deficiency may modify the presence of sucking pests (SADEJ, SADEJ 2001). *Aphis fabae* aphid, as one of few species, has been the subject of investigations on insect requirements for minerals. It was stated that *Aphis fabae* for its development requires the presence of major quantities of Mg and small amounts of Fe, Mn, Zn and Cu in the liquid feed it takes (DAAD 1967, 1968). The content of these elements under conditions of soil polluted with petrol was considerably decreased. The four-fold higher lead content in the object where soil was polluted with 3 g kg<sup>-1</sup> of petrol (PI) could not have been the factor limiting *A. fabae* fecundity in the second generation nor its intrinsic rate of natural increase in a population (Table 2). The experiments on the influence of soil contamination with this element on pest biology on broad bean did not reveal its effect in the first generation, even at an eight-fold higher Pb content in the aerial parts, whereas even slightly higher fecundity was observed in the second generation than on unpolluted plants, receiving mineral fertilisation (GOSPODAREK 2012). Although the content of 1.39 mg Pb dm<sup>-3</sup> reduced fecundity and the rate of population development in *Brevicoryne brassicae* aphid on radish and cabbage by about 30% (GORUR 2007), this is a much higher dose of the metal than found in even strongly polluted environments. On the other hand, fecundity and longevity of *Aphis nerii* on the oleander tree was negatively correlated with lead concentrations in leaves at its relatively low content, 1.3, 4.0, 7.1 and 9.2 ppm (GHABEISH 2014), which suggests differences depending on the host plant species and the herbivore.

Table 2

Biological parameters of black bean aphid *Aphis fabae* Scop. on broad bean growth in oil derivatives polluted soil

Details	$d$		$M_d$		$r_m$	
	I	II	I	II	I	II
Control	7.647a*	7.583a	24.64c	15.86b	0.300b	0.268d
PI	8.300a	8.000a	21.85bc	12.04a	0.275ab	0.229bc
PII	7.938a	8.167ab	21.96abc	13.59ab	0.282ab	0.235bc
DFI	8.133a	9.000c	20.70abc	10.44a	0.279ab	0.194a
DFII	7.905a	9.200c	17.84a	13.60ab	0.265a	0.213ab
EOI	7.695a	8.000a	19.09ab	16.69b	0.285ab	0.253cd
EOII	8.000a	8.857bc	19.59ab	13.90ab	0.275ab	0.217ab

$d$  – days to reproduction,  $M_d$  – mean number of larvae born in time =  $d$ ,  $r_m$  – intrinsic rate of natural increase in population;

\* Means followed by the same letters in columns did not differ significantly at  $\alpha = 0.05$  according to the LSD test; see Fig. 1 for symbols.

Considerable inhibition of black bean aphid development due to the soil contamination with Ni was found at its concentrations in broad bean plants several dozen times higher than in unpolluted plants (GOSPODAREK 2012). On the other hand, aphid fecundity did not change on broad bean plants where the cadmium concentration was elevated by about five-fold in comparison with unpolluted plants. In the present investigations, an increased (by about 70%) Ni content was only determined in the object where soil was contaminated with the higher dose of petrol, whereas the Cd level in PII and DFII objects was similar to the one detected in the control plants of the aforementioned experiment.

The life span of wingless females of the first generation was from 22.40 to 30.41 days, while in the second generation it ranged from 18.21 to 22.75 (Table 1). Both in the first and the second generation, the females feeding on broad beans growing in soil contaminated with the engine oil dose equal 3 g kg<sup>-1</sup> lived the longest, which corresponds to their high fecundity and also to the demonstrated high nitrogen concentration in broad beans. For the first generation, a shorter female life span was observed in the objects contaminated with the higher doses of petrol and engine oil, but the differences were significant only for the objects where aphids lived the longest (i.e. EOI and DFI), whereas none of the objects revealed any marked difference in comparison with the control. Also, in the investigations concerning the effect of increasing Zn doses (0.25 mg dm<sup>-3</sup>, 1 mg dm<sup>-3</sup>, 5 mg dm<sup>-3</sup> and 10 mg dm<sup>-3</sup> of the medium) on development parameters of *A. fabae*, the life span proved to be a feature far less susceptible to this element's impact than fecundity (GOSPODAREK 2005). Research on the use of invertebrates, such as springtails (Collembola) and earthworms (Lumbricidae), as bioindicators of soil contamination

tion with oil derivatives also point to fecundity as a feature far more useful than the survival rate in an assessment of ecological risk (EOM et al. 2007).

Aphid larvae needed from 7.647 to 8.300 days to reach the reproductive period in the first generation and from 7.583 to 9.200 days in the second generation (Table 2). Both in the first and second generation, aphids feeding on unpolluted plants revealed the shortest pre-reproductive period. In the first generation, no significant effect of the soil pollution on the duration of the pre-reproductive period was noticed, whereas in the second generation a significant extension of that time was found for aphids feeding on plants in the soil polluted with diesel fuel (irrespective of the dose) and with the higher dose of engine oil. DAVIES et al. (1998) stated that the average time to reach sexual maturity by *A. fabae* aphid larvae on broad bean under laboratory conditions was  $5.85 \pm 0.35$  days. On the other hand, the said period in a study on the effect of heavy metals on *A. fabae* development was between 5.7 and 12.2 days, depending on the object and generation (GOSPODAREK 2012). A longer pre-reproductive period in the present investigations might have been due to the experiment being conducted in greenhouse conditions. CRAWFORD et al. (1995) demonstrated the lengthening of the pre-reproductive period from 8.1 to 10.1 days and diminishing of fecundity from 35.6 on the control to 23.5 larvae per *A. fabae* female on broad bean plants exposed to cadmium dosed at  $50 \mu\text{g ml}^{-1}$  of the medium, which led to the cadmium concentration in plants between 63.5 to  $456 \mu\text{g Cd g}^{-1}$  after the experiment finished.

An average number of larvae produced by wingless females during the time equal to the pre-reproductive period ( $M_d$ ) in the first generation was the highest in the control. A significant decrease in this parameter was registered under the influence of the contamination with the higher dose of diesel fuel and engine oil (irrespective of the dose),  $F = 2.257$ ,  $P = 0.043$ . On the other hand, in the second generation, the females feeding on the PI and DFI objects gave birth to the fewest larvae (markedly fewer than the control) in this period.

The intrinsic rate of natural increase in a population ( $r_m$ ) in females from the control was 0.300 for the first generation and 0.268 for the second generation, thus being the highest among all the objects. A significant decline in this parameter's value in the first generation was documented only under the influence of the soil pollution with the higher dose of diesel fuel, whereas in the second generation  $r_m$  decreased in response to all the applied pollutants except for the lower dose of engine oil ( $F = 6.77$ ,  $P < 0.0001$ ). The soil pollution with petrol reduced  $r_m$  less than did diesel fuel or the higher dose of engine oil. As has been indicated in literature, a negative effect of petrol on living organisms lasts much shorter than that of diesel fuel and engine oil because of evaporation can largely lower the content of the former in polluted soil (KIREEVA et al. 2005). The intrinsic rate of natural increase in a population for black bean aphid on broad bean is 0.345 acc. to DOUGLAS (1997) and 0.359 acc. to FRAZER (1972). On the other hand, in the research on the impact of soil contamination with heavy metals on black bean aphid development on broad bean, the value of this parameter for aphids feeding on



unpolluted plants and without mineral fertilisation was 0.306 (on average for 6 subsequent generations), whereas on plants receiving mineral fertilisation it decreased to 0.248 (on average for 8 subsequent generations), although similarly to the current research,  $r_m$  of the second generation was lower than that of the first generation (GOSPODAREK 2012).

Comparably to our results, GORUR (2007) found slightly higher values of  $r_m$  parameter in *B. brassicae* aphids feeding on unpolluted plants, as compared with the ones exposed to lead and copper, but the differences were not statistically proven.

Summing up, most of the doses of the investigated oil derivatives caused a decrease in most of the analysed macro- and microelements in broad bean leaves, but contributed to an elevated content of such heavy metals as lead and cadmium (at the higher dose of oil derivatives). The above changes in the host plants might have deteriorated development parameters of *Aphis fabae* Scop., especially the ones determined in the 2<sup>nd</sup> generation. The study provided information about potential negative effects of oil derivatives on further links of the food chain, i.e. for predators and parasitoids. Similar, far reaching effects of pollutants have been described in literature (WINDER et al. 1999, GHABEISH 2014).

However, no negative effect on the development of aphids was observed for the lower dose of engine oil, and – notably – the fertility observed in the 1<sup>st</sup> generation of aphids feeding on plants grown in this variant was even better, which provides information about a potential negative effect of such slight pollution with engine oil (typically encountered) on plant production due to possibly greater harmfulness of this pest.

## CONCLUSIONS

1. Soil pollution with petrol dosed at 3 g kg<sup>-1</sup> led to a significant decrease in the content of Ca, Mg, Fe, Mn, Zn, Ni and Cu in the aerial parts of broad bean, while causing a marked increase in the Pb content. The double petrol dose additionally contributed to diminishing the N content, whereas the Ni and Cd concentrations increased significantly after its application.

2. Soil pollution with diesel fuel, irrespective of the dose, caused a significant decline in the Ca, N, Zn, Ni and Cu concentrations and an increase in the Pb content. Additionally, the dose of 6 g kg<sup>-1</sup> led to an increase in the Mg, Fe and Cd content in broad bean plants.

3. Soil pollution with used engine oil dosed at 3 g kg<sup>-1</sup> led to a reduction of the Ca, Mg, Fe, Mn, Zn and Cu content in broad bean shoots, simultaneously causing an increase in Pb and a slight increase in N concentrations. The double dose of engine oil caused a decline only in the Ca, Mn, Ni and Cu content.

4. The effect of oil derivatives on the biology of black bean aphid was more pronounced in the second generation. All the analysed substances, except for engine oil in the lower dose, significantly decreased the intrinsic rate of natural increase in a population. The lack of negative effect of the low ( $3 \text{ g kg}^{-1}$ ) dose of engine oil may be connected with the nitrogen content in plants (slightly elevated in comparison with the control and other objects with polluted soil). Soil pollution with diesel fuel affected development parameters of black bean aphid more than pollution with petrol did.

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